

Load of MDV DNA in peripheral blood as criterion for early diagnosis of Marek's disease

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Abstract

Outbreaks of Marek's disease (MD) in vaccinated flocks occur sporadically leading to economic losses. In previous work, we have demonstrated that high load of MDV DNA in tumors is a good diagnostic criterion but it does not permit an early diagnosis of MD. In this study we have evaluated if high load of MDV DNA in peripheral blood could aid in the early diagnosis of MD. A series of experiments combining various MD vaccines and challenge viruses were conducted to simulate field conditions. Samples of blood were taken periodically and gross lesions were evaluated at the end of the experiments. Our results show that chickens that developed MD by the end of the experiment had high load of MDV DNA as early as 3 week post inoculation. Differences in the load of MDV DNA between chickens with tumors and chickens without tumors were more remarkable when measured in whole blood than in buffy coats. The use of this criterion to monitor vaccine efficacy is discussed.

Introduction

Monitoring of Marek's disease protection in the field is extremely difficult because Marek's disease virus (MDV) is ubiquitous and infection is not synonymous with disease. Moreover, even though there is strong neutralizing antibody response after MD vaccination, neutralizing antibodies do not protect against development of tumors. Therefore they cannot be used to estimate the level of protection conferred by MD vaccine. We have recently shown that amount of MDV DNA present in MD-induced tumors is a very useful criteria to diagnosis MD. Islam *et al.* have shown that chickens that develop tumors had higher levels of MDV DNA in buffy coats as early as 2-3 weeks post infection (Islam *et al.*, 2006). The objectives of this work were (1) to determine if the load of MDV DNA in peripheral blood is correlated with the presence of tumors, (2) to compare the load of MDV DNA in peripheral blood and in buffy coats, and (3) to determine if amount of MDV DNA in peripheral blood can be used as a criteria to monitor protection conferred by vaccines.

Materials and Methods

Chickens. Chickens were Marek's disease-susceptible F1 progeny (15X7) of Avian Disease and Oncology Laboratory line 1515 males and line 71 females. They were from unvaccinated breeder hens, free of antibodies to MDV and HVT as well as other common poultry pathogens.

Real Time PCR. Real time PCR assay was performed as previously described (Gimeno *et al.*, 2003).

Experimental design. One experiment was conducted using a careful combination of vaccines and oncogenic MDV (HVT/GA, HVT/Md5, HVT/648A, HVT+SB1/Md5, HVT+SB1/648A, GA, Md5 and 648A) that provide different level of protection (Table 1). Vaccines were administered subcutaneously at hatch at a dose of 2000 PFU. Challenge with oncogenic virus was conducted 5 days post vaccination, by the subcutaneous route, at a dose of 500 PFU. Samples of blood were collected at 3, 5 and 15 weeks post challenge (wpc) and load of MDV DNA was evaluated from whole blood and buffy coat. Details of the experimental groups and virus strains used are shown in Table 1.

Statistical analysis. Data were analyzed with the statistical program Statistica (Stat Soft, Tulsa, OK, USA). Comparisons among groups were conducted by an analysis of variance (ANOVA) test. The level of significance considered was $P \leq 0.05$.

Table 1. Experimental design

Lot	Level protection ¹	Vaccine			Challenge			% Expected tumors ²
		Strain	Dose (PFU)	Age	Strain	Dose	Age	
1	p	HVT	2000	1d	GA	500	6d	<5%
2	np	HVT	2000	1d	Md5	500	6d	>50% ²
3	np	HVT	2000	1d	648A	500	6d	>90%
4	p	HVT+SB1	1000+1000	1d	Md5	500	6d	<25%
5	np	HVT+SB1	1000+1000	1d	648A	500	6d	>50%
6	NA	-----	-----	-----	-----	-----	-----	0%

¹ Level of protection, p (protected) and np (non protected) is based on the level of expected tumors.

² Percentage of expected tumors are based on the results of previous studies (REF)

Results

MDV DNA load by treatment groups. Percentage of MD lesions varied in each of the treatment group. Protected groups (HVT/GA and HVT+SB-1/Md5) had the lowest percentage of chickens with MD lesions. In each treatment groups, chickens showing MD lesions had higher MDV DNA load in peripheral blood (Figure 1)

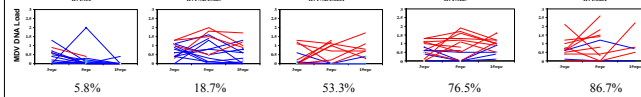


Figure 1. MDV DNA load in whole blood of chickens vaccinated and challenge with various viruses at different time points (3, 5 and 15 weeks post challenge, wpc). Blue lines indicate the MDV DNA load in chickens with no MD tumor at the end of the study (15 wpc). Red lines indicate the MDV DNA load in chickens with MD tumors at the end of the study. Percentage of chickens that develop MD tumors in each group is indicated below each graphic

MDV DNA load by lesion groups. When considering all treatment groups, chickens that developed tumors also had higher load of MDV in both whole blood and buffy coats that chickens that did not develop tumors. Differences were statistically significant as early as 3 wpc and tended to increase at 5 wpc and at 15 wpc (Figure 2). Load of MDV DNA in buffy coats was higher than in peripheral blood. However, differences in the load of MDV DNA between chickens with tumors and chickens without tumors were more remarkable when measured in whole blood than in buffy coats.

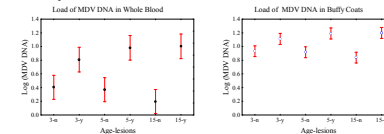


Figure 2. MDV DNA load in whole blood and buffy coats at different time points (3, 5, and 15 wpc) of chickens that developed (y) or not (n) tumors by the end of the study (15wpc). MDV DNA load is expressed as log of the number of MDV DNA copies per 50 ng of total DNA. Chickens from all treatment groups were classified as positive (y) and negative (n) for tumors, including all the treatment groups, and results are presented as the average and standard error.

MDV DNA load by protection groups. MDV DNA load in peripheral blood of chickens belonging to the better protected groups (HVT/GA, HVT+SB-1/Md5) was lower than those belonging to the less protected groups (HVT+SB1/648A, HVT/Md5, HVT/648A) at all time points (Figure 3). However, differences were statistically significant only at 15 wpc

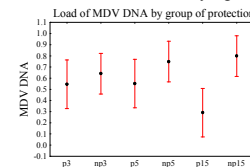


Figure 3. MDV DNA load in whole blood on chickens belonging to the protected (p) groups (HVT/GA and HVT+SB1/Md5) and to the non protected (np) groups (HVT+SB1/648A, HVT/Md5, HVT/648A) at the different time points (3, 5, and 15 wpc). MDV DNA load is expressed as log of the number of MDV DNA copies per 50 ng of total DNA. Results are presented as the average and standard error.

Discussion

In this work we have used a model to simulate field conditions, in which chickens with different level of vaccine protection are exposed to MDVs of different virulence. Using this model, we have shown that chickens that develop tumors has higher load of MDV DNA in both whole blood and buffy coats, and differences can be detected as early as 3 wpc. This finding supports the results of Islam *et al.* that reported higher MDV DNA in buffy coats of chickens that develop tumors (Islam *et al.*, 2006). Our results showed that measurement of MDV DNA in whole blood is advantageous versus measurement of MDV DNA in buffy coats. Besides of the obvious technical advantages, differences in the load of MDV DNA between chickens with tumors and chickens without tumors were more remarkable when measured in whole blood than in buffy coats.

An early detection of chickens that will develop tumors is extremely relevant not only in research but also in the field to monitor the efficiency of vaccination. With this purpose we test if the criterion of MDV DNA load in blood will be valid to differentiate groups of chickens well protected by MD vaccines *versus* those not well protected. Our results showed that, although there is a tendency, statistically significant differences could be detected at 15 wpc but not at 3 or 5 wpc. These results indicate that MDV DNA load in blood is a good criterion for diagnosis Marek's disease in a flock and also to predict if a chicken is going to develop MD tumors under research conditions. However, it might not be sensitive enough to be used to predict the development of a MD outbreak in the field. Further studies are warranted to evaluate this criterion using other combinations of MD vaccine and challenge virus, and also under field conditions.

References

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For further information

Please contact Isabel_Gimeno@ncsu.edu. More information on this and related projects can be obtained at <http://www.msu.edu/~arsadol> and at <http://www.cvm.ncsu.edu/dph/plm/Personnel/gimeno.html>